d-Cycloserine facilitates extinction of a cocaine-induced conditioned place preference

Fanny Botreau, Giovanna Paolone, Jane Stewart

Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, SP-A-244, 7441 Sherbrooke Street West, Montreal, Que., Canada H4B 1R6

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Abstract
d-Cycloserine, a partial NMDA agonist, significantly accelerated extinction of a cocaine-induced conditioned place preference (CPP) when rats were given systemic injections immediately, but not 4 h, after each extinction trial. Infusions directly into the basolateral amygdala had a similar effect. The facilitative effect of d-cycloserine on the extinction of appetitive conditioning is consistent with the idea of the formation of new learned associations during extinction.

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Extinction of conditioned responses based on aversive or appetitive unconditioned stimuli (US) is used as a therapeutic measure to reduce fears or cravings [2,5,17,18,31]. For example, the reduction of conditioned fear involves the repeated presentation of the conditioned fear stimulus (CS) in the absence of the US (an extinction procedure). Similarly, the reduction of conditioned cravings or appetites can be brought about by repeated presentation of the conditioned appetitive stimulus (CS) in the absence of a US, such as food or the injection of a drug of abuse.

Interestingly, there is ample evidence that extinction does not erase the original conditioned associations, but rather involves new learning in new circuits that inhibits the original conditioned response [13,21]. Thus, the learning of and memory for extinction of conditioned responses might be expected to involve neurochemical processes similar to those involved in the original learning of the association. For these reasons, in attempts to enhance or facilitate extinction processes and to improve the retention of this new learning, researchers have investigated the same glutamatergic receptor mechanisms known to be involved in conditioning and learning, and, in particular, those at the NMDA receptor (for initial learning, [4,9,14]; for extinction, [3,16,24]).

Recently, it was shown in a series of experiments that extinction of conditioned fear responses can be facilitated by injections of the partial NMDA glutamatergic receptor agonist, d-cycloserine (d-4-amino-3-isoxazolidinone), a compound that acts at the strychnine-insensitive glycine-recognition site of the NMDA receptor complex, and which does not produce any obvious neurotoxicity in rats [7,30]. With the knowledge that NMDA receptor antagonists can attenuate the acquisition of extinction [2,8], Walker et al. [29] studied the effect of d-cycloserine on extinction using a fear-potentiated startle procedure. They found that pre-trial injections of d-cycloserine given either systemically or directly into the basolateral amygdala promote extinction of conditioned fear. Subsequent experiments confirmed these results by showing a similar facilitative effect of d-cycloserine on extinction of conditioned freezing when intra-peritoneal or intra-amygdala injections took place either before or immediately after extinction sessions [11]. However, when administration of d-cycloserine after each extinction session was delayed, the facilitative effect decreased linearly, and was no longer effective after a delay of 4 h. These authors suggest that d-cycloserine acts on acquisition and/or consolidation of extinction memories, whose processes occur shortly after the trial [11] (for review, see [23]).

To date, all of the published studies of the effect of d-cycloserine on extinction processes have been carried out using aversive conditioning. Here, we report on its effects on extinction
after the last conditioning trial, rats were placed in the center conditioning and was blocked by guillotine doors. Two days after the last conditioning trial, rats were placed in the center choice area (15.5 cm × 7.5 cm) by allowing the rat access to the entire apparatus in a drug-free state. A CPP is demonstrated if the rat spends a greater amount of time in the drug-paired than in the vehicle-paired compartment. Subsequently, the preference can be reduced or eliminated by giving repeated CPP tests in the drug-free state (extinction trials), a method that allows for the observation of the extinction of the preference over days [15]. In Experiment 1, we studied the effect of systemic injections of d-cycloserine on extinction of the CPP given to rats immediately after each session. In Experiment 2, rats were given the injections of d-cycloserine 4 h after each extinction session, when in studies of extinction of aversive conditioning it was found not to affect extinction, presumably because it was given after the period of consolidation of new learning (extinction). In Experiment 3, we studied the effect of d-cycloserine on the original learning of the CPP, by injecting it immediately after each session of conditioning, to see whether if by enhancing the original conditioning, it would subsequently increase trials to extinction. Finally, in Experiment 4, the involvement of the basolateral amygdala (BLA) in the facilitation of the formation of new associations during extinction was examined by injecting d-cycloserine directly into this nucleus immediately after every other session on three occasions. Moreover, long-term consequences of the facilitative effects were studied in a final test for CPP given 2 weeks after the last extinction session.

Male Long-Evans rats (300–350 g; Charles River, Canada) were housed individually in a humidity- and temperature-controlled colony room on a reverse 12 h light–dark cycle (lights off at 8 h), with food and water at all times. All experimental procedures were approved by the Animal Care Committee of Concordia University, in accordance with the guidelines of the Canadian Council on Animal Care.

The conditioned place preference task was conducted during the dark phase of the cycle in four identical gray PVC plastic rectangular boxes composed of two distinctive conditioning compartments (24 cm × 35 cm) separated from a smaller center choice area (15.5 cm × 19.5 cm) by guillotine doors. After 3 days of handling, rats were placed in the center choice chamber with the guillotine doors removed and allowed free access to the entire apparatus for 15 min. The amount of time spent in each chamber during this CPP test was recorded to assess individual preferences. No injections were given during the CPP test, maintaining the same procedure as that used during the pre-exposure test. To induce extinction of this cocaine-paired compartment preference, similar CPP tests were conducted over the following days. The effect of d-cycloserine on extinction was studied by injecting rats with d-cycloserine or saline intraperitoneally immediately after each session of extinction (15 mg/kg, dissolved in a 0.9% saline from Sigma Oakville, Canada; n = 24; Experiment 1). In a subsequent experiment, d-cycloserine or saline was injected 4 h after each session of extinction (in the colony room; n = 10; Experiment 2). The d-cycloserine effect on initial learning was investigated by injections immediately after each session of conditioning (n = 12; Experiment 3). In a final experiment rats were implanted with bilateral guide cannulae under pentobarbital anesthesia (Somnotol, 65 mg/kg, i.p. Maple Leaf Foods, Cambridge, ON) aimed above the BLA for subsequent infusions directly into the structure (AP = +2.5 mm; ML = +4.8 mm; DV = −6.2 mm; 10 µg/0.5 µl/site, 0.25 µl/min) through 28 gauge injection cannulae. Rats were then trained on the CPP as described and infusions of d-cycloserine or saline were given immediately after the 1st, 3rd and 5th of eight extinction sessions. Rats were retested 2 weeks after the last extinction session (n = 18; Experiment 4).

Pre-exposure and CPP tests outcomes were determined by time spent in each chamber. The effect of Chamber was analysed by within-subjects repeated measures ANOVAs followed by Student–Newman–Keuls post hoc comparisons of the time spent in the two conditioning chambers on each session of extinction. Differences were considered statistically significant at p < 0.05.

In Experiment 1, during pre-exposure, rats spent more time in the chamber that was associated with cocaine during conditioning (see Fig. 1; F(2,44) = 24.39, p < 0.001); but the time in the outer compartments did not differ. During the first CPP test after conditioning, rats spent more time in the chamber that was associated with cocaine during conditioning (see Fig. 1; F(2,44) = 82.57, p < 0.001) and showed a significant preference for the cocaine-paired chamber over both the other chambers (p < 0.001). Fig. 1 shows time spent in the two outer compartments over extinction trials. It can be seen that when d-cycloserine was injected immediately after the first CPP test and after all subsequent extinction sessions, the preference decreased more rapidly than when saline injections were given. For the ANOVA, the extinction data were collapsed into three 3-day blocks (also for Experiments 2 and 3). Since the time spent in the center compartment did not change during the entire period of extinction sessions, analyses were restricted to saline- and cocaine-paired compartments. The ANOVA revealed significant main effects of Block (F(2,44) = 11.59, p < 0.001), and Chamber (F(1,22) = 17.88, p < 0.001) and a significant interaction between Treatment and Block (F(2,44) = 4.89, p < 0.05) that reflected the faster decrease of the preference for the cocaine-paired chamber in d-cycloserine-treated rats. Post hoc analyses revealed that the preference for the cocaine-paired chamber was significant for the first 8 days of extinction in saline-treated rats,
Fig. 1. Extinction of cocaine-induced CPP in rats receiving saline or d-cycloserine intraperitoneally (saline: n = 11; d-cycloserine: n = 13) immediately after each session of extinction. Mean (±S.E.M.) time spent in cocaine-paired and saline-paired chambers in daily 15-min tests for CPP. d-Cycloserine or saline were given after the first CPP test and after all subsequent extinction sessions. *p < 0.05; **p < 0.01; ***p < 0.001.

whereas in d-cycloserine-treated rats there was no preference after day 3 of extinction, except on days 8 and 10.

In Experiment 2, rats again spent a greater amount of time in the cocaine-paired chamber than in the saline-paired chamber during the first CPP test (see Fig. 2). F(2,18) = 35.15, p < 0.001) and post hoc analyses indicated that the time spent in the cocaine-paired compartment was significantly greater than in the other two chambers (p < 0.01). During extinction, when d-cycloserine was injected 4 h after each extinction session, there was no facilitation of extinction over that seen in the saline-treated group. The ANOVA revealed a significant effect of Chamber (F(1,9) = 31.20, p < 0.001), without any effect of Treatment (F(1,9) < 1, ns).

In Experiment 3, when rats were injected with d-cycloserine or saline immediately after each conditioning session, both groups showed a preference for the cocaine-paired chamber (see Fig. 3). F(2,20) = 27.23, p < 0.001), but there was no effect of treatment (F(1,10) < 1, ns) and no interaction between these two factors (F(2,20) < 1, ns). It can be seen in Fig. 3 that during extinction sessions the preference for the cocaine-paired chamber decreased gradually in both groups, but there was no clear difference between groups. ANOVA revealed a significant effect of Chamber (F(1,10) = 38.40, p < 0.001), but no interaction between Treatment and Block (F(2,20) = 3.44, ns). Post hoc comparisons showed that in saline-treated rats the preference for the cocaine-paired chamber lasted until the 6th extinction ses-

Injections immediately after each extinction session

![Injections immediately after each extinction session](image)

Fig. 2. Extinction of cocaine-induced CPP in rats receiving saline (n = 5) or d-cycloserine (n = 6) 4 h after each session of extinction. Mean (±S.E.M.) time spent in cocaine-paired and saline-paired chambers in daily 15-min tests for CPP. d-Cycloserine or saline were given after the first CPP test and after all subsequent extinction sessions. *p < 0.05; **p < 0.01; ***p < 0.001.

Injections 4 hours after each extinction session

![Injections 4 hours after each extinction session](image)
Fig. 3. Extinction of cocaine-induced CPP in rats that had received d-cycloserine (n=6) or saline (n=6) immediately after each session of conditioning. Mean (±S.E.M.) time spent in cocaine-paired and saline-paired chambers in daily 15-min tests for CPP. The first CPP test measured the initial cocaine-paired chamber preference. No injections were given after this initial test or throughout extinction.

Fig. 4. Extinction of cocaine-induced CPP in rats receiving saline or d-cycloserine directly into the BLA (Saline: n=6; d-cycloserine: n=8) immediately after each extinction session. Mean (±S.E.M.) time spent in cocaine-paired and saline-paired chambers in daily 15-min tests for CPP. d-Cycloserine or saline were given after the 1st, 3rd and 5th sessions of extinction. *p<0.05, **p<0.01, ***p<0.001.
injections were delayed is similar to that found previously in aversive conditioning [11]. In accordance with evidence that extinction involves new learning and does not erase the original conditioned association [13,21], these experiments suggest a facilitative action of \( \alpha \)-cyclotylerine on neural processes that are ongoing immediately after the experience of the extinction session and are involved in the consolidation of the memory for extinction conditions. There is one previous experiment, however, in which the effect of \( \alpha \)-cyclotylerine was studied after training of lever pressing for food [20]. In this experiment, the drug was injected after a "memory" test given under extinction conditions and was found to increase responding. It should be noted, however, that \( \alpha \)-cyclotylerine was given before this test and that its effects on the course of extinction were not studied.

Interestingly, two studies on drug-induced conditioned reward have shown that a time-limited facilitation of extinction can be obtained using other treatments after extinction sessions. It has been shown that post-extinction injections of either oxotremorine, a cholinergic muscarinic receptor agonist, or glucose, facilitate extinction of an amphetamine-induced CPP [25,26]. Moreover, this facilitative effect on extinction is not observed when oxotremorine and glucose injections are delayed by 2 h. Taken together, these studies support the idea that drugs known to affect consolidation of conditioning also affect consolidation of memory for extinction [12,24,25].

We also showed in these experiments that the facilitative effect of \( \alpha \)-cyclotylerine given peripherally could be replicated by local injections directed into the BLA. These data concerning the involvement of the BLA in extinction of appetitive responses are consistent with the finding that excitotoxic lesions of the BLA made after learning of a cocaine-induced CPP retard the subsequent extinction of the preference [6]. The importance of BLA in processes mediating the extinction of learned responses has also been seen in studies of facilitation of extinction of aversive conditioning by \( \alpha \)-cyclotylerine [11,29] and of appetitive conditioning by cholinergic muscarinic receptor agonist [25,26].

Taken together, these findings suggest that NMDA, as well as cholinergic, receptors located in the basolateral amygdala play a key role in processes facilitating consolidation of memory for extinction of both aversive and appetitive conditioned responses.

An important finding from our study of the effects of infusions of \( \alpha \)-cyclotylerine into the BLA is that there was complete loss of the CPP after only three extinction trials and two infusions of \( \alpha \)-cyclotylerine given after the first and third trials. Interestingly, the extinction of the preference seen after three extinction trials was evident in tests made 2 weeks after the end of extinction sessions, indicating a long-lasting effect of \( \alpha \)-cyclotylerine treatment. The finding that the effects of \( \alpha \)-cyclotylerine are evident after few \( \alpha \)-cyclotylerine treatments is similar to that seen in experiments on aversive conditioning (e.g. [11]).

Finally, although \( \alpha \)-cyclotylerine has been shown to affect initial learning of responses, we found no effect on the magnitude of the conditioned place preference when \( \alpha \)-cyclotylerine was injected after each session of conditioning. We had reason to expect that post-trial injections of \( \alpha \)-cyclotylerine might enhance the initial learning of the CPP, but on the first test the magnitude of the preference for the cocaine-paired compart-

ment was similar in \( \alpha \)-cyclotylerine- and saline-treated rats. The lack of effect of \( \alpha \)-cyclotylerine as measured by the first CPP test might be explained by a ceiling effect; the dose of cocaine used during the conditioning might have been such that the CPP could not be enhanced by any treatment. Another possibility is that the learning that takes place during extinction may be more fragile, or less robust, than that occurring during the initial conditioning. Thus, consolidation of extinction may be easier to modulate (to facilitate or to interrupt) than that of the initial conditioning. A third possible explanation is that previous demonstrations of the involvement of NMDA receptors in place preference conditioning were made using NMDA antagonists, and not agonists, administered before conditioning sessions [1,10,19,22,27]. Consequently, the effects of NMDA receptor antagonists observed in these experiments could have been due to interference with the encoding of events during conditioning and not to interference with consolidation processes.

In summary, we have found that \( \alpha \)-cyclotylerine, recently shown to accelerate extinction of conditioned fear, also facilitates extinction of a conditioned appetitive response. \( \alpha \)-cyclotylerine apparently acts by enhancing the consolidation of memories of extinction conditions, processes are, at least in part, mediated by NMDA receptors in the basolateral amygdala. It is well known that conditioned stimuli previously associated with drugs of abuse can induce craving and relapse in humans. It is possible therefore, that \( \alpha \)-cyclotylerine, or similar agents, could be used to help human drug addicts to extinguish the emotional responses and thoughts induced by environments and cues previously associated with drug use. As a result, these findings may contribute to the development of pharmacological therapies to avoid or at least reduce craving in humans during the periods of detoxification and abstinence.

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References


